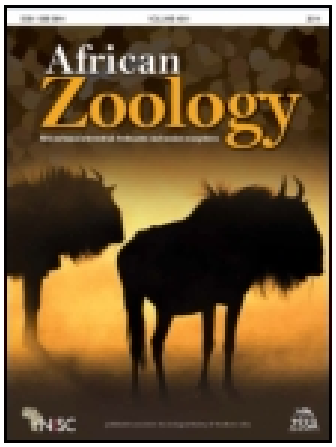

African Zoology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tafz20

Population dynamics and biology of an invasive population of mosquitofish Gambusia affinis in a temperate estuarine lake system

Hans Sloterdijk[ab], Nicola C James[b], M Kyle S Smith[c], Werner Ekau[a] & Olaf LF Weyl[bd]

[a] Leibniz Center for Tropical Marine Ecology, Bremen, Germany

[b] South African Institute for Aquatic Biodiversity, Grahamstown, South Africa

[c] South African National Parks, Rondevlei Scientific Services, Sedgefield, South Africa

[d] Centre for Invasion Biology, South African Institute for Aquatic Biodiversity, Grahamstown, South Africa

Published online: 02 Apr 2015.

To cite this article: Hans Sloterdijk, Nicola C James, M Kyle S Smith, Werner Ekau & Olaf LF Weyl (2015) Population dynamics and biology of an invasive population of mosquitofish Gambusia affinis in a temperate estuarine lake system, African Zoology, 50:1, 31-40

To link to this article: http://dx.doi.org/10.1080/15627020.2015.1021169

African Zoology 2015, 50(1): 31–40

AFRICAN ZOOLOGY
ISSN 1562-7020 EISSN 2224-073X
http://dx.doi.org/10.1080/15627020.2015.1021169

Population dynamics and biology of an invasive population of mosquitofish *Gambusia affinis* in a temperate estuarine lake system

Hans Sloterdijk[1,2], Nicola C James[2], M Kyle S Smith[3], Werner Ekau[1] and Olaf LF Weyl[2,4]*

[1] *Leibniz Center for Tropical Marine Ecology, Bremen, Germany*
[2] *South African Institute for Aquatic Biodiversity, Grahamstown, South Africa*
[3] *South African National Parks, Rondevlei Scientific Services, Sedgefield, South Africa*
[4] *Centre for Invasion Biology, South African Institute for Aquatic Biodiversity, Grahamstown, South Africa*
* *Corresponding author, email: o.weyl@saiab.ac.za*

This study investigates the distribution, relative abundance, population growth and biology of the alien invasive fish *Gambusia affinis* in the Wilderness Lakes System, a coastal lake system in the warm-temperate region of South Africa. Fish were collected throughout the system during four seasonal sampling trips in spring (October 2010), summer (February 2011), autumn (May 2011) and winter (July 2011). *Gambusia affinis* were widespread and abundant in all habitats from the freshwater reaches of the inflowing Touw River to the more saline lakes and estuary. Sex ratio was generally female dominated and mean length at maturity was 14.7 mm standard length (SL) for males and 20.3 mm SL for females. Reproduction was strongly seasonal with reproductively active fish sampled in spring and summer but not in autumn and winter. Relative abundance also varied seasonally, with populations following typical 'boom and bust' population dynamics. By winter, the population in all habitats was lower with mortality rates as high as 85%. The stronghold of the population appears to be in the channels between the lakes, where relative abundance was more consistent and winter mortality rates were lower. The survivors, which are primarily females, then reconstitute the population in the spring and summer months.

Keywords: alien, clutch size, length at maturity, life history, mortality rate, relative fecundity, reproduction, South Africa

Introduction

The western mosquitofish *Gambusia affinis* Baird and Girard, 1853 is a small (40 mm standard length), viviparous freshwater fish native to south-eastern North America that has been widely introduced into fresh and brackish waters around the world (Pyke 2008). In wild populations, longevity seldom exceeds 12–15 months (Pyke 2005, 2008), during which females are able to produce multiple clutches ranging from five to over 100 young (Pyke 2005). The species is able to tolerate a wide range of physical conditions, including temperatures from 4 to 44 °C (Jobling 1981; Nordlie 2006) and salinities up to 41 g kg^{-1} (Chervinski 1983; Pyke 2005, 2008; Uliano et al. 2010), but generally prefer shallow, slow-flowing water that is densely vegetated (Pyke 2008). *Gambusia affinis* are opportunistic omnivores and their diet includes algae, crustaceans, insects, amphibian larvae and small fishes including conspecifics (Dionne 1985; Meffe and Crump 1987; Daniels and Felley 1992; Leyse et al. 2004). These life-history traits are generally associated with successful invaders (Marchetti et al. 2004) and, as a result, *G. affinis* have established populations on all continents except Antarctica (Pyke 2008). Together with the closely related *Gambusia holbrooki* Girard, 1859, they are the most widely distributed freshwater fish in the world (Pyke 2005).

While humans have often regarded these introductions as beneficial because of their control of mosquitoes (Pyke 2008), their predation on and competition with native biota have been shown to have significant impacts on recipient ecosystems (Hurlbert et al. 1972; Pyke and White 2000; Pyke 2005; Alcaraz and García-Berthou 2007). As a result, *G. affinis* is listed among the world's worst invasive fish species (Lowe et al. 2000). In South Africa, *G. affinis* were formally introduced in 1936 (De Moor and Bruton 1988) and, as a result of introductions for mosquito control and as a fodder fish for introduced sportfishes, the species has become established in many river systems and is considered fully invasive (Ellender and Weyl 2014). Invasions in the Cape Floristic Region, a global biodiversity hotspot (Linder et al. 2010), are of particular concern because the native fish fauna in this region is already highly impacted by human activities, including pollution, habitat alteration and catchment degradation (Tweddle et al. 2009). In areas where other anthropogenic impacts are limited, competition with and predation by alien fishes is considered a primary threat to native freshwater biota (Tweddle et al. 2009).

Monitoring and control of alien invasive species is a legislated priority for managers of protected areas in South Africa (Foxcroft and McGeoch 2011; Spear et al. 2011). To be effective, however, monitoring and control programmes require knowledge of the distribution, population dynamics and life-history characteristics of the species of concern (Spear et al. 2011). While the biology of *G. affinis* is generally

well understood (see reviews by Pyke 2005, 2008), there are few quantitative studies that describe the population dynamics of *G. affinis* in large natural systems (Howell et al. 2013). The recently reported *G. affinis* invasion of the Wilderness Lakes System, located in the Garden Route National Park in the Western Cape of South Africa (Olds et al. 2011), provided the opportunity to investigate an invasive *G. affinis* population in a relatively pristine, large estuarine lake system. The objective of this study was to describe and quantify the biology, distribution, relative abundance and population growth of *G. affinis* in the Wilderness Lakes System, in order to assess its invasion status and to assess the feasibility of management actions.

Materials and methods

Study area

The Wilderness Lakes System (33°59′–34°00′ S, 22°36′–22°43′ E) is located in the warm-temperate biogeographic region of South Africa (Figure 1) and covers an area of approximately 13 km² (Randall 1990). It is one of only a few coastal lake systems in the country and is an important component of the Garden Route National Park under the jurisdiction of South African National Parks. The system comprises the temporarily open/closed Touw estuary and a series of three coastal lakes (Eilandvlei, Langvlei and Rondevlei) that are connected by shallow channels (Figure 1). The mouth of the estuary is open for approximately 28% of the time (Russell 2013). In the Wilderness Lakes System, the native fish community consists entirely of obligate catadromous and euryhaline marine and estuarine species, while non-native fishes are all of freshwater origin (Olds et al. 2011). *Gambusia affinis* were first reported from the inflowing Touw and Duiwe rivers in 1997 (Russell 1999) and from the estuary and lake system in 2010 (Olds et al. 2011).

As a result of its location within a protected area, the water quality and aquatic plant distribution of the Wilderness Lakes System have been monitored since 1991 (see Russell 2013). Water temperature follows seasonal fluctuations, with the highest water temperatures occurring in December and January (summer) and the lowest in July (mid-winter). Over a 20-year period (1991–2010) the temperature range in the Touw Estuary was 8.2–27.2 °C, and between 11.0 and 27.4 °C in the lakes (Russell 2013). Salinity is variable; the Touw Estuary exhibits a typical longitudinal salinity gradient, with salinities at the mouth being dependent on its connectivity to the marine environment. The lakes frequently exhibit a reversed salinity gradient, with salinity increasing towards Rondevlei, the lake furthest from the sea (Russell 2013). Turbidity in the system is moderate (average below 10 NTU) (Russell 2013).

The lakes and channels are bordered by a narrow margin of emergent aquatic plants including *Juncus kraussii*, *Paspalum vaginatum*, *Cotula coronopifolia* and *Sarcocornia* spp. with *Phragmites australis* being the most prominent. Submerged aquatic macrophytes are widespread in all of the water bodies and consist predominantly of pure and mixed stands of *Potamogeton pectinatus*, Chlorophyta and filamentous algae (*Cladophora* spp.), while *Zostera capensis* occurs sporadically in the Touw Estuary (Howard-Williams and Liptrot 1980; Weisser and Howard-Williams 1982; Whitfield et al. 1983). The channel habitat is characterised by well-developed beds of submerged vegetation, with a more-or-less continuous band of submerged macrophytes in relatively shallow water. In contrast, the lake habitat is

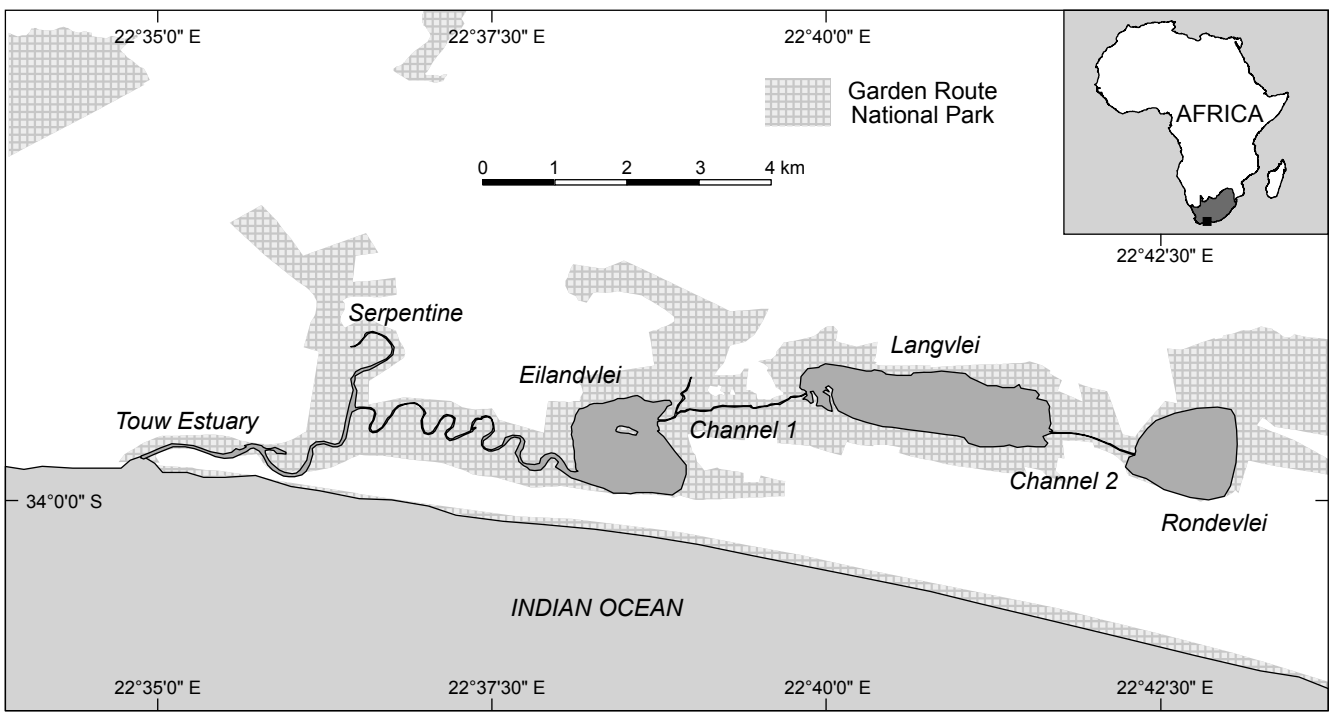

Figure 1: Map of the Wilderness Lakes System, South Africa. The shaded area demarcates the Garden Route National Park

typically more heterogeneous, with a deeper area of lower density and lower variety vegetation.

Fish sampling

Gambusia affinis were collected during four seasonal sampling events in spring (October 2010), summer (February 2011), autumn (May 2011), and winter (July 2011) at 68 sampling sites (Touw Estuary $n = 6$; Serpentine channel $n = 10$; Eilandvlei $n = 11$; Langvlei $n = 16$; Rondevlei $n = 10$; interconnecting channel 1 $n = 6$; channel 2 $n = 9$), which were selected to attain spatial representation along the shallow littoral zone of the water bodies that comprise the Wilderness Lakes System (Figure 2).

Fishes were sampled using a scoop net with a 2 mm mesh size and a hoop diameter of 75 cm. At each sampling site, five haphazard sweeps were conducted with the scoop net from the surface to just above the benthos. Each sweep covered a distance of approximately 3 m and care was taken not to sample the same area twice. Surface water temperature and salinity (grams per kilogram) were measured at selected sampling sites using a hand-held multi-parameter water analyser (Model 30, YSI, Inc., Yellow Springs, OH, USA).

All fishes caught were identified to species level and counted. All *G. affinis* were retained for biological analysis, and immediately euthanised with an overdose of the anaesthetic benzocaine hydrochloride in accordance with the South African Institute for Aquatic Biodiversity and South African National Parks standard operating procedures. Thereafter, they were preserved in 10% buffered formaldehyde, with specimens from different water bodies being kept separate.

Relative abundance

Assessments of relative abundance follow those used by Howell et al. (2013). Recorded counts of *G. affinis* captured per sweep were used to investigate relative abundance between sampling sites over the sampling period. Relative abundance was measured as catch per unit effort (CPUE), which was expressed as the number of fish per scoop. The CPUE data were square root transformed to meet assumptions of normality and homoscedasticity. Based on microhabitat similarities, in terms of depth and vegetation cover, sites were grouped into three habitats: (1) estuary (includes the Touw Estuary and Serpentine), (2) lake (Eilandvlei, Langvlei and Rondevlei) and (3) channels (interconnecting channels 1 and 2). The effects of the continuous variables time (days from the first sample), temperature, salinity and the categorical variable habitat in controlling *G. affinis* CPUE were tested using a general linear model (GLM). These factors represented potentially important drivers of *G. affinis* population dynamics, which variance inflation analysis within the GLM showed to be orthogonal.

A repeated-measures ANOVA was used to test the differences in *G. affinis* CPUE between habitats and over sequential sampling events, to assess whether or not a 'boom–bust' pattern of rapid population growth and

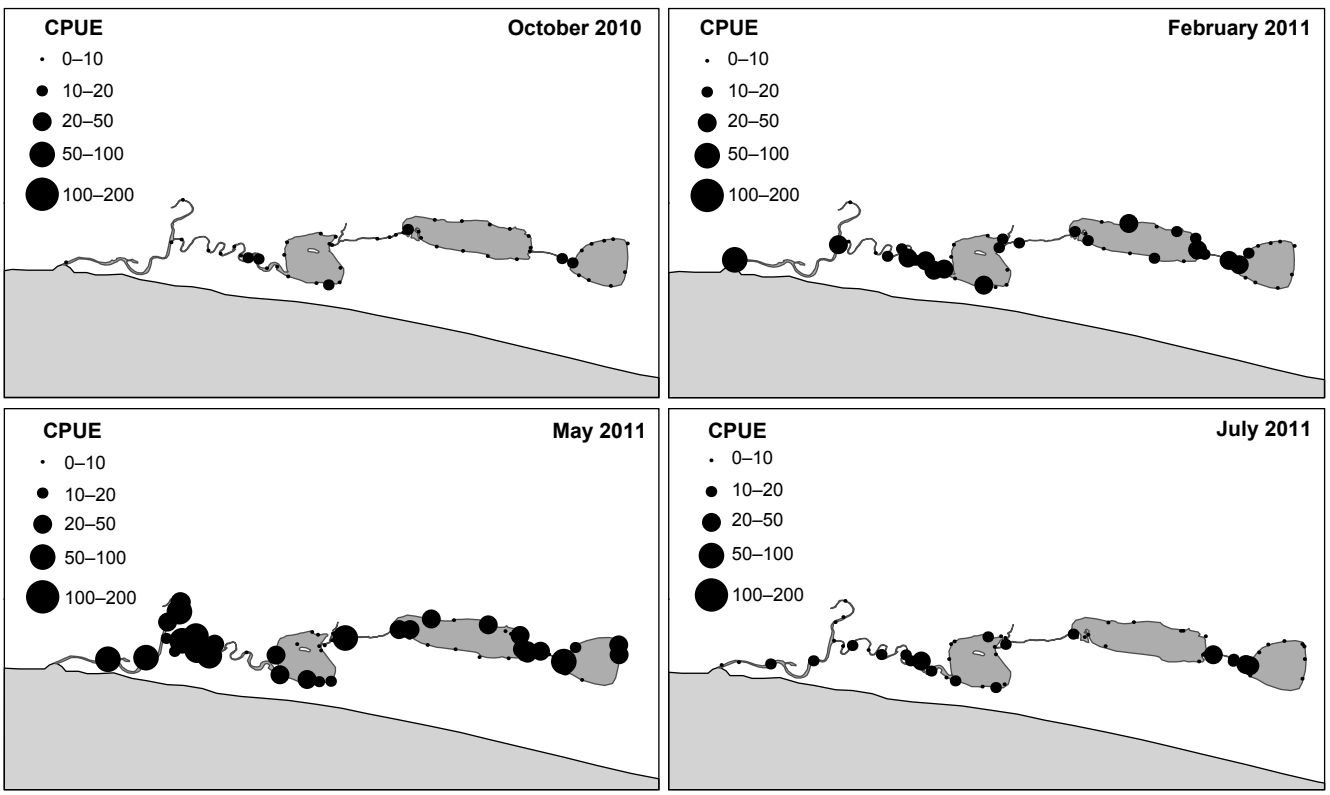

Figure 2: Spatial distribution of catch per unit effort (CPUE; fish per scoop) of *Gambusia affinis* in scoop net samples in the Wilderness Lakes System over four sampling events between October 2010 and July 2011

subsequent decline occurred in each of the three habitats. Significant differences in CPUE between individual sampling events were then examined using Tukey *post hoc* tests.

The average daily rate of increase of *G. affinis* CPUE was used to further compare the relative population growth patterns between habitats across the sampling period. The average rate of increase of *G. affinis* per scoop per day (within each habitat) was calculated using the difference in CPUE between adjacent sampling events (for each habitat) and dividing that value by the number of days that had occurred between the two sampling events. The timing of key inflection points in the population dynamics of the three populations (estuary, lakes and channels) were then assessed graphically.

Laboratory procedures

All fish were washed and gradually transferred from 10% to 50% and 70% ethanol for preservation. All fish were measured for standard length (SL) to the nearest 0.05 mm and a sample of 906 fish was measured for both SL and total length (TL) to derive the morphometric relationship between SL and TL. The relationship between SL and total weight (TW) was determined from 4 824 fish. Large fish (SL > 14 mm) were weighed to the nearest 0.01 g and small fish (SL < 14 mm) to the nearest 0.00001 g. Relationships between SL and TL and SL and TW were described by linear regression and non-linear regression (power curve), respectively.

Sex ratio and population structure

All individuals were sexed by morphological observation under a dissecting microscope in accordance with the criteria presented in Table 1. Chi-square (χ^2) contingency tables were used to assess whether sex ratio was dependent on habitat (2 sexes × 3 habitats) in each of the four seasons sampled. Population structure by length was assessed graphically and the dependence of the proportion of juvenile and mature male and female fish on season was tested using a χ^2 contingency table (3 categories × 4 seasons).

Maturity

Maturity was assessed visually for 4 805 individuals collected during the reproductive season (October and February). Mature males were distinguished from immature males on the basis of the development of the gonopodium, whereas female maturity was assessed by dissection and visual inspection of the ovaries for signs of embryonic development as described in Table 2. Length at first maturity (L_S) was the smallest mature individual of either sex. Length at 50% maturity (L_{M50}) was determined by fitting a logistic model to the proportion of reproductively mature fish in 1 mm size classes using the formula $P(L_S) = 1/[1 + \exp^{(L_S - L_{M50})/\phi}]$, where $P(L_S)$ is the proportion of mature fish (ES 0, ES 1, ES 2 and ES 3 specimens for females, and specimens with fully developed gonopodium for males) at length L_S and ϕ is the width of the ogive curve.

Table 1: Criteria used to sex the juvenile, male and female *Gambusia affinis* sampled from the Wilderness Lakes System. SL = standard length

Sex	Maturity	Description
Juvenile	Undetermined	Individual < 14 mm (SL) with no gonopodium
Male	Immature	Presence of under-developed gonopodium
	Mature	Presence of fully developed gonopodium
Female	Immature	Individual > 14 mm (SL) with no gonopodium
	Mature	Individual > 14 mm (SL) with mature, unfertilised or fertilised oocytes apparent in the ovaries

Reproductive period

Reproductive period was determined on the basis of visual assessment and the calculation of a relative fecundity index (RFI). Visual assessments involved scoring the stage of ovarian and embryonic development in potentially reproductive female fish ($L_S > L_{M50}$) collected in spring (October), summer (February), autumn (May) and winter (July). To estimate RFI, fish were dissected and oocytes and embryos were counted after removal of the intra-ovarian tissue. After dissection, fish were weighed to obtain eviscerated weight (W_E) and RFI was estimated as $RFI = N_E/W_E$, where N_E represents the sum of mature oocytes and embryos (Haynes and Cashner 1995). To assess for seasonal differences, RFI for females exceeding L_{M50} were grouped by season and compared using a Kruskall–Wallis non-parametric ANOVA. Dunn's all-pairwise method was used for multiple comparison.

Results

Physicochemical parameters

The mouth of the estuary was closed in October, February and May and open during sampling in July. Surface water

Table 2: Criteria used to distinguish the stages of ovarian and embryonic development in female *Gambusia affinis* (adapted from Trendall 1982). ES = embryo stage, SL = standard length

Stage	Description
Ovarian development	
Immature	Ovaries small and white. No sign of vitellogenesis in oocytes
Resting	Individual > 14 mm SL with no gonopodium and characterised by the absence of mature oocytes and embryos
Embryonic development	
ES 0 (Gravid)	Mature, unfertilised oocytes, and newly fertilised oocytes where embryonic development is not apparent. Translucent yellow/orange with many marked oil droplets clearly visible
ES 1	From fertilised oocytes showing streak or groove to embryo with eyes apparent, but somatic development indistinct
ES 2	From ES 1 to stage where embryo completely encircles yolk sac and caudal fin is in contact with head
ES 3	From ES 2 until birth. Yolk sac small or indistinct. Somatic development near completion

temperature varied little across the system (Table 3) and was highest in summer (mean ± SD: 27.0 ± 0.3 °C) and lowest in winter (mean ± SD: 14.3 ± 0.3 °C). A reverse salinity gradient was present in October, February and May, with the highest salinities recorded in Langvlei and the Rondevlei/Langvlei Channel (Channel 2). When the estuary mouth was open in July, a normal horizontal salinity gradient was present, with the highest salinities (29.5 g kg^{-1}) recorded in the mouth region of the Touw Estuary (Table 3).

Relative abundance

A total of 19 563 *G. affinis* were counted during relative abundance surveys. The contribution of other species to net samples was less than 2.5% and therefore considered incidental. *Gambusia affinis* were captured at 84% of the sample sites, indicating that CPUE estimates were not biased by high numbers of zero catches. CPUE at individual sites for the four sampling periods is shown in Figure 2.

The results of the GLM showed that CPUE was significantly determined by time (days from first sample: df = 1; $F = 25.3$; $p < 0.0001$), temperature (df = 1; $F = 50.8$; $p < 0.05$) and habitat (df = 2; $F = 15.6$; $p < 0.0001$), but not by salinity (df = 1; $F = 1.1$; $p = 0.29$). Repeated measures ANOVA results demonstrated that CPUE also varied significantly among habitats (df = 2, 48; $F = 20.1$; $p < 0.0001$) and across sampling events (df = 3, 144; $F = 55.1$; $p < 0.0001$), with a significant interaction between habitat and sampling event also evident (df = 6, 144; $F = 5.2$; $p < 0.0001$). This result shows that, while the *G. affinis* population size rose and fell significantly in all sites over the year, the magnitude of both population increases and decreases varied from habitat to habitat (Figure 3). Despite these variations in population dynamics, an overall trend of rapid increase, plateau, and then severe decline in density in the final winter sample was evident across all habitats (Figure 3).

When CPUE was expressed as an average daily rate of increase (Table 4) it is evident that populations increased most rapidly in the estuary, followed by the channel and then the lake habitat. For example, over the 117 d period from the spring to the summer sample, CPUE increased at an average rate of 0.11 fish $scoop^{-1}$ d^{-1} and ranged from 0.06 fish $scoop^{-1}$ d^{-1} in the lakes to 0.15 fish $scoop^{-1}$ d^{-1} in the estuary. During the 89 d between the summer and autumn samples, CPUE continued to increase in all habitats except in the channel (Table 4). Subsequently, during the progression from autumn to winter after Day 206, populations in all sites expressed negative population growth rates, indicating marked declines in population size.

Biology

Morphometrics

For male, female and combined sexes of *G. affinis* sampled from the Wilderness Lakes System, the relationships between TL and SL was best described as: male TL (mm) = 1.1251 × SL (mm) + 1.5069 ($r^2 = 0.95$, df = 354); female TL (mm) = 1.1468 × SL (mm) + 1.0852 (r^2 =0.99; df = 438) and combined sex TL (mm) = 1.1616 × SL(mm) + 0.7041 ($r^2 = 0.99$; df = 905). Weight at length was best described by the relationship TW (g) = 0.000004 × SL $(mm)^{3.3969}$ ($r^2 = 0.98$, df = 4 823).

Sex ratio

In total 6 527 fish were sampled for sex ratio during this study. Of these 4 251 were female and 2 276 were male. Sex ratio, however, differed between habitats and seasons. In October, sex ratios were significantly dependent on habitat ($\chi^2 = 79.1$, df = 2, $p < 0.001$), with populations in the channel habitat being strongly female dominated (female/male = 3.14) while those in lake (0.83) and estuary (0.72)

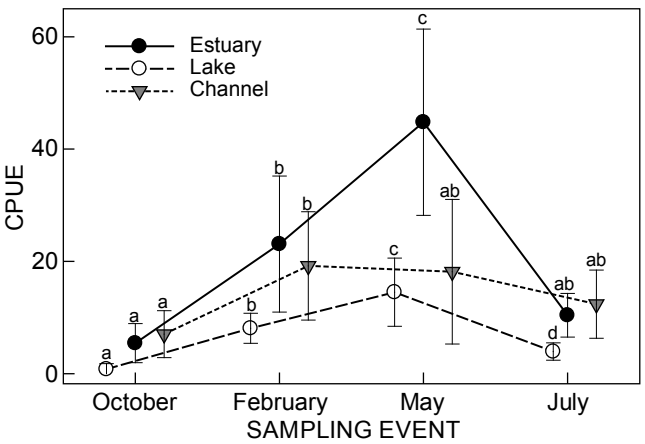

Figure 3: Mean catch per unit effort (CPUE; fish per scoop) of *Gambusia afffinis* in scoop net samples collected between October 2010 and July 2011 from three habitats in the Wilderness Lakes System, South Africa. Error bars represent the 95% CI. Different letters above the error bars denote statistically significant differences (Tukey's test, $p < 0.05$)

Table 3: Mean (± SD) temperature and salinity in the Wilderness Lakes System during four sampling events from October 2010 to July 2011

Locality	Temperature (°C)				Salinity (g kg^{-1})			
	Oct	Feb	May	Jul	Oct	Feb	May	Jul
Estuary mouth	19.0 ± 0.0	25.0 ± 0.0	14.5 ± 0.0	13.2 ± 0.0	4.3 ± 0.0	6.7 ± 0.0	5.2 ± 0.0	29.5 ± 0.0
Touw Estuary	18.8 ± 0.1	25.3 ± 0.2	15.1 ± 0.1	14.2 ± 0.1	3.0 ± 1.2	6.2 ± 0.6	1.7 ± 1.8	8.5 ± 5.3
Serpentine	24.5 ± 2.6	29.4 ± 1.6	15.7 ± 0.4	14.2 ± 0.1	3.2 ± 2.3	3.0 ± 1.1	6.9 ± 0.1	6.3 ± 0.5
Eilandvlei	19.2 ± 0.3	25.1 ± 0.1	16.3 ± 0.2	14.0 ± 0.9	9.4 ± 0.0	7.9 ± 0.0	7.0 ± 0.0	5.7 ± 0.4
Channel 1	22.7 ± 0.4	27.5 ± 1.1	14.6 ± 1.1	12.4 ± 2.6	8.9 ± 0.1	8.5 ± 0.3	7.8 ± 3.8	6.1 ± 4.8
Langvlei	19.8 ± 0.1	26.8 ± 0.2	13.3 ± 0.9	15.5 ± 1.4	10.2 ± 0.0	10.4 ± 0.0	10.3 ± 0.4	9.0 ± 0.5
Channel 2	21.5 ± 0.5	26.9 ± 0.3	13.6 ± 0.9	15.7 ± 0.0	10.3 ± 0.1	10.2 ± 0.2	9.0 ± 1.4	9.7 ± 0.0
Rondevlei	20.4 ± 0.0	26.6 ± 0.2	15.2 ± 0.6	15.2 ± 0.2	9.0 ± 0.0	9.5 ± 0.0	9.6 ± 1.1	9.3 ± 0.8

habitats were male dominated (Figure 4). By February, sex ratios were still dependent on habitat ($\chi^2 = 33.8$, df = 2, $p < 0.001$) but all habitats were now female dominated, with females increasing in proportion to males in lake and estuary habitats and decreasing in the channel habitat. Sex ratio equalised between habitats in May ($\chi^2 = 4.3$, df = 2, $p > 0.1$) and July ($\chi^2 = 1.5$, df = 2, $p > 0.4$), but remained strongly female dominated at 3.86 females per male in May and 2.73 females per male in July (Figure 4).

Maturity

A total of 1 425 male and 1 879 female *G. affinis* collected during the reproductive period (October and February) were used for estimating length and maturity (Figure 5). The smallest mature male was 12.6 mm SL, L_{M50} was estimated at 14.7 mm SL and all fish were mature at 20 mm SL. For females, the smallest mature fish sampled was 17.3 mm SL, L_{M50} was attained at 20.3 mm SL and all fish were mature at 25 mm SL.

Reproductive period

Strong seasonality was observed in the distributions of gonadal (immature) and embryonic developmental stages (ES 0 to ES 3) (Figure 6a). Females in all gonadal and embryonic conditions were encountered in both October and February, while in May only two female fish containing embryos (ES 0) were sampled and only five fish containing embryos (ES 3) in July (Figure 6b). Similarly, the relative fecundity index for mature females differed between seasonal sampling events ($P < 0.05$; Figure 6a). Median RFI decreased significantly from 54.5 embryos fish^{-1} in October to 0 embryos in May and July. Mean (± SD) clutch size, of fish containing ES 3 embryos, was 18.7 ± 12.4 (range = 1–63, $n = 82$).

Population structure

The proportion of adult male, female and juvenile fish was significantly dependent on season (3 categories × 4 seasons contingency table, $\chi^2 = 676$, df = 6, $P < 0.0001$). In spring, the population is primarily comprised of adults (75%), with large (> 28 mm SL) adult females (37%) and smaller (> 18 mm SL) adult males (38%) dominating the population (Figure 7). Spring recruitment is indicated by a well-defined cohort of small juvenile fish (< 14 mm SL), comprising 25% of the total population. From summer onwards, the population was dominated by juveniles, which comprised 56% of the population in summer, 57% in autumn and 52% in winter. Mature male and female components of the population were predominantly small individuals (> 14 mm SL) (Figure 7).

Table 4: Rate of increase of *G. affinis* catch per unit effort recorded from October 2010 to July 2011 in three habitats in the Wilderness Lakes System, South Africa

Month	October	February	May	July
Temperature (°C)	20.7	26.6	14.8	14.3
Day	0	117	206	267
Rate (fish scoop^{-1} d^{-1})				
Estuary	0	0.15	0.24	−0.56
Channel	0	0.10	−0.01	−0.09
Lakes	0	0.06	0.07	−0.17
Average	0	0.11	0.10	−0.27

Discussion

The introduction pathway for *G. affinis* into the Wilderness Lakes System is not known, although it has been present in adjacent catchments since 1972 (de Moor and Bruton 1988) and their spread was most likely facilitated by an informal introduction into the catchment. The current assessment demonstrates that *G. affinis* is a highly successful invader in the Wilderness Lakes System with specimens sampled throughout the system during all seasons (Figure 2). In a similar study conducted in the Sundays River Valley Irrigation System, *G. affinis* was present but at lower densities (Howell et al. 2013). The high numbers of *G. affinis* recorded in the Wilderness

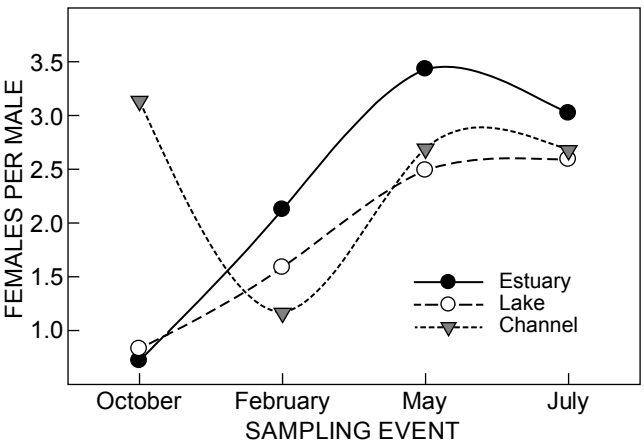

Figure 4: Sex ratio of *Gambusia affinis* in three major habitats over four sampling events conducted between October 2010 and July 2011 in the Wilderness Lakes System, South Africa

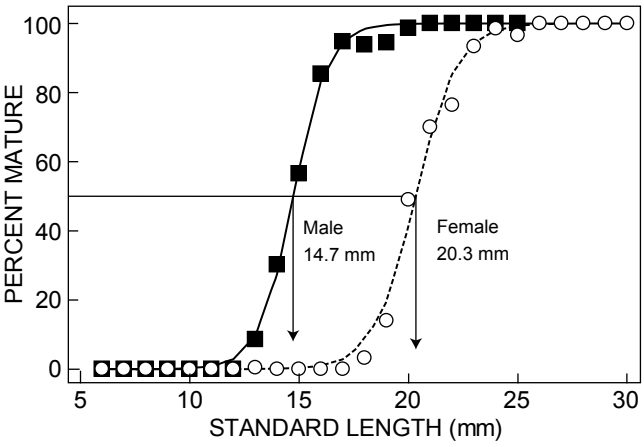

Figure 5: Proportion of mature male (filled squares, $n = 1\ 425$) and female (open circles, $n = 1\ 879$) by 1 mm standard length (SL) classes for *Gambusia affinis* sampled from the Wilderness Lakes System. The fitted logistic maturity ogive and lengths at 50% maturity are indicated for each sex

Lakes System indicate that they are well adapted to its warm-temperate climate and moderate salinity. Furthermore, the slow-moving water and densely vegetated margins conform to documented habitat preferences of this species (Casterlin and Reynolds 1977; Miura et al. 1979).

The reproductive season of *G. affinis* has been linked to environmental conditions, with both temperature and photoperiod being key factors in the onset of the reproductive cycle (Vondracek et al. 1988; Pen and Potter 1991; Fraile et al. 1994). With a gestation period of generally 22–35 d (Krumholz 1948; Lloyd 1986), the presence of young of the season and females carrying embryos of stage ES 3 in the October samples, indicate that the breeding season had begun towards the end of September or the beginning of October (early spring), when temperatures become warmer and the photoperiod starts to exceed 12 h. The early start in the reproductive season could allow for a potential five to six broods during the six-month breeding season.

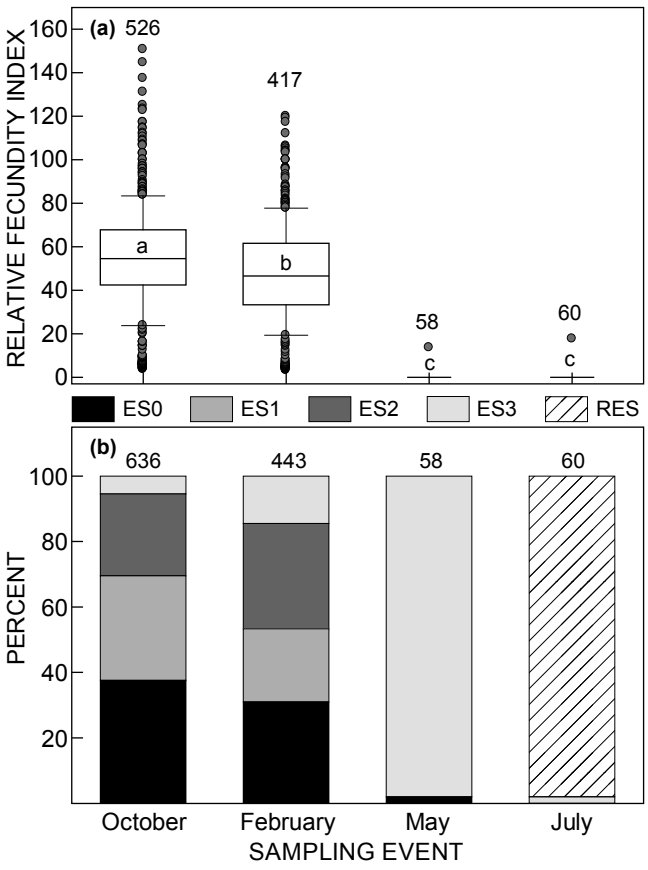

Figure 6: (a) Relative fecundity index and (b) percentage of female *Gambusia affinis* per macroscopic maturity stage between October 2010 and July 2011 in the Wilderness Lakes System, South Africa. Boxplots indicate the median, and 25th and 75th percentiles. Error bars indicate the 5th and 95th percentiles, and outliers are shown as data points. Numbers above the bars are the sample sizes. Different letters above the box-plot medians denote significant differences ($p < 0.05$). RES = resting, ES0 = mature oocytes where embryonic development is not apparent, ES1 = fertilised oocytes, ES2 = embryo completely encircles yolk sac, ES3 = embryo with somatic development near completion

Relative abundance varied seasonally, and populations followed typical 'boom and bust' population dynamics, with the highest CPUE generally recorded in autumn (May) at the end of the breeding season, and thereafter CPUE decreased into winter. There are few documented studies on mortality rates in wild *Gambusia* populations (Pyke 2008), but mortality rates of between 20% and 50% have

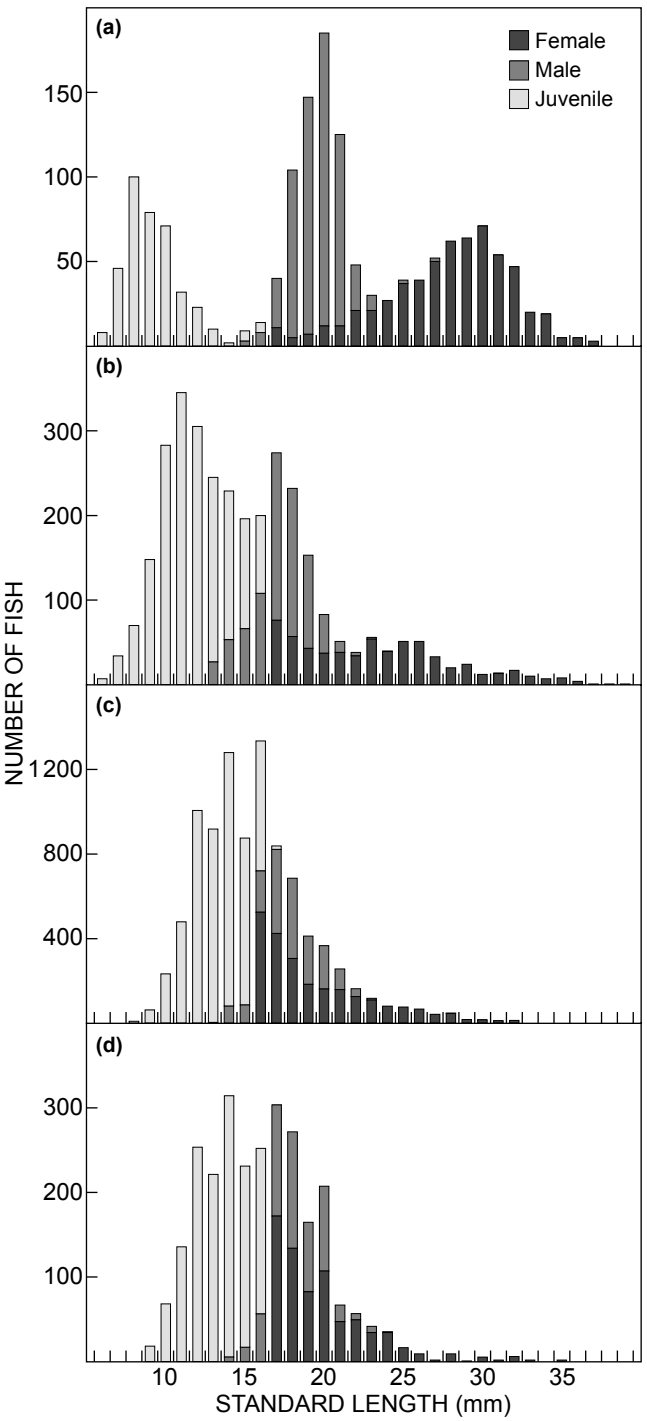

Figure 7: Frequency of juvenile, male and female *Gambusia affinis* in 1 mm size classes in the Wilderness Lakes System sampled in (a) October 2010, (b) February 2011, (c) May 2011 and (d) July 2011

been recorded for laboratory populations over periods of approximately three months (Stearns and Sage 1980). Pyke (2008) stated that mortality was likely to be much higher under less benign natural conditions and Haynes (1993) reported that mortality is higher during winter than in warmer months and may be as high as 99%. Measured mortality rates (inferred by decreased CPUE) in the Wilderness Lakes System were of this order of magnitude, with populations decreasing between 37% and 85% by the middle of winter.

Gambusia affinis is a eurythermal species, with a reported lower lethal temperature limit of approximately 0 °C (Cherry et al. 1976). Over a 20-year period, the water temperature in the Wilderness Lakes System has ranged between 8.2 and 27.2 °C in the Touw Estuary and between 11.0 and 27.4 °C within the lakes (Russell 2013). With the lowest recorded temperature being well above the lower lethal temperature for *G. affinis*, the thermal regime of the system should not, in isolation, result in mortality of this species. However, the onset of winter and prolonged exposure to low temperatures may significantly slow down their metabolic activity, leading to increased susceptibility to disease and predation by piscivorous birds and fish. In addition, the cessation of breeding and concomitant lack of recruitment over autumn and winter exacerbate rapid mortality. Water temperature was found to be the only physicochemical variable that significantly determined CPUE. However, the onset of the breeding season should also be considered as a confounding variable and the observed variation of CPUE attributed in part to reproductive effort, which in turn is influenced by water temperature and photoperiod.

Although *G. affinis* is able to survive in a wide variety of environmental conditions (Pyke 2005), certain habitat types promote survival of this species during unfavourable times of the year. In the Wilderness Lakes System, this is likely to be the case during the winter months. In October, very low CPUE values were obtained for the lake habitat, whereas higher values were recorded in the channel habitat. In contrast, in February when conditions were favourable, high CPUE values were recorded in all habitats. The differences recorded in CPUE between habitats may be related to predation pressure or different habitat characteristics. For example, Olds et al. (2011) used seine nets to sample for larger species and found that channel habitats contained no piscivorous species, whereas the estuary and lake habitats contained piscivorous predators including *Monodactylus falciformis*, *Lichia amia* and *Galeichthys feliceps*. Moreover, the channel habitat may offer protection from wind, leading to less water movement than in the lake habitat and perhaps more stable water temperatures, particularly in shallow areas.

The population structure of *G. affinis* varied over the sampling period, with the population consisting of two main annual cohorts, with a cohort substitution during the reproductive season. Similar results have been recorded for other populations of *G. affinis* (Krumholz 1948; Trendall 1982; Hughes 1985; Fernández-Delgado 1989; Fernández-Delgado and Rossomano 1997; Howell et al. 2013). The majority of individuals forming the population appear to die during the winter months (June–August), with survivors reproducing and reconstituting the population in the spring and summer months (October–March). This premise is supported by the dominance of post-winter adults in October and their decline and replacement by young of the season (sex-undetermined and immature) in February.

Although roughly equal numbers of both sexes are known to occur at birth (Krumholz 1948; Haynes and Cashner 1995), wild populations of *G. affinis* are normally female dominated (Snelson 1989) and females form the majority of individuals that survive to another year (Pérez-Bote and López 2005). In the Wilderness Lakes System, males dominated the lake and estuary habitats in October, while the population in the channels was strongly female dominated. By the end of the study (July) the population in all habitats was strongly female dominated. A possible explanation for the observed sex ratios is that mature gravid females move from channel populations to colonise and repopulate areas where they occur in lower numbers. According to Barney and Anson (1921), recruitment and colonisation occurs mostly during the breeding season. Garcia-Berthou (1999) reported that in Lake Banyole (north-east Spain), females are more numerous, except in spring when males are more common. Alternatively, the observed male dominance in certain habitats during October in the lake habitat may be related to selective predation on larger female *G. affinis*, a phenomenon that has been observed in other studies (Maglio and Rosen 1969; Britton and Moser 1982).

Gambusia affinis is a sexually dimorphic species (Bisazza and Pilastro 1997). Females continue to grow throughout their life, which allows them to maximise their reproductive output, whereas males stop growing shortly after reaching sexual maturity (Vondracek et al. 1988) and small males are genetically maintained in most poeciliid fishes (Bisazza and Pilastro 1997). This was also the case in the Wilderness Lakes System, where females were generally larger than males (Figure 7). Length at maturity for both males and females was within the 13–24 mm SL range reported by Pyke (2005).

Maximum RFI was recorded in October, at the beginning of the reproductive period, with females reaching their greatest average size. Given that the RFI is a function of female length and clutch size, these results were to be expected. Most females caught in October bred for the first time in their second breeding season, having probably been born near the end of the previous breeding season. Other studies have shown that these females have larger clutches than females that breed in the same season in which they were born (Krumholz 1948; Maglio and Rosen 1969; Haynes and Cashner 1995).

Gambusia affinis is well established in the Wilderness Lakes System and is common and abundant in all habitats from the freshwater reaches of the inflowing Touw River to the more saline lakes and the estuary. While the stronghold of the population appears to be in the channels between the lakes, where relative abundance was more consistent and winter mortality rates were lower, populations in the lakes and estuary grow rapidly even from low population sizes. In the Wilderness Lakes System *G. affinis* has increased its range and according to the unified framework for invasive species proposed by Blackburn et al. (2011) can be classified as a fully invasive species. Blackburn et

al. (2011) suggest containment, mitigation and eradication as appropriate management measures for fully invasive species. Unfortunately, eradication is impractical because the species is able to reproduce from very low population sizes (e.g. in the lakes) and mitigating against further spread by attempting to contain the *G. affinis* population may be the only viable management option. Faced with a similar scenario when dealing with an armoured catfish *Pterygioplichthys disjunctivus* (Weber 1991) invasion in the Richards Bay area in South Africa, Jones et al. (2013) proposed an education campaign to inform the general public of the dangers associated with fish introductions to mitigate against further spread. While such an education campaign would not benefit the Wilderness Lakes System directly, it may help to prevent other invasions of this and other invasive fish species. In addition, impacts of *G. affinis* in South African ecosystems are not documented to date and we recommend that the ecological role of this species in the Wilderness Lakes System be investigated to more fully understand its potential impacts.

Acknowledgements — This research project would not have been possible without the collaboration and support of the Leibniz Center for Tropical Marine Ecology (ZMT, Bremen, Germany), the South African Institute for Aquatic Biodiversity (SAIAB, Grahamstown, South Africa) and the South African National Parks (Rondevlei Scientific Services, Sedgefield, South Africa). The DST/NRF Centre of Excellence for Invasion Biology (Stellenbosch, South Africa) and the National Research Foundation of South Africa (NRF) are thanked for their financial contributions. Our most genuine indebtedness is owed to the Wilderness section staff of the Garden Route National Park in South Africa for sharing their time, knowledge, enthusiasm and resources during this project. We would also like to thank the Rhodes University Department of Ichthyology and Fisheries Science, BSc Honours class of 2011 and Alexis Amy Olds (now at CapeNature) for field assistance. The comments of two anonymous reviewers improved this manuscript and are appreciated.

References

Alcaraz C, García-Berthou E. 2007. Life history variation of an invasive fish (*Gambusia holbrooki*) along a salinity gradient. *Biological Conservation* 139: 83–92.

Barney RL, Anson BJ. 1921. Seasonal abundance of the mosquito destroying top-minnow, *Gambusia affinis*, especially in relation to male frequency. *Ecology* 2: 53–69.

Bisazza A, Pilastro A. 1997. Small male mating advantage and revised size dimorphism in poeciliid fishes. *Journal of Fish Biology* 50: 397–406.

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V et al. 2011. A proposed unified framework for biological invasions. *Trends in Ecology and Evolution* 26: 333–339.

Britton RH, Moser ME. 1982. Size specific predation by herons and its effect on the sex-ratio of natural populations of mosquito fish *Gambusia affinis* (Baird and Girard). *Oecologia* 53: 146–151.

Casterlin ME, Reynolds WW. 1977. Aspects of habitat selection in the mosquitofish *Gambusia affinis*. *Hydrobiologia* 55: 125–127.

Cherry DS, Guthrie RK, Rodgers JH, Cairns J, Dickson KL. 1976. Response of mosquitofish (*G. affinis*) to ash effluent and thermal stress. *Transactions of the American Fisheries Society* 105: 686–694.

Chervinski J. 1983. Salinity tolerance of the mosquito fish, *Gambusia affinis* (Baird and Girard). *Journal of Fish Biology* 22: 9–11.

Daniels GL, Felley JD. 1992. Life history and foods of *Gambusia affinis* in two waterways of southwestern Louisiana. *South Western Naturalist* 37: 157–165.

de Moor IJ, Bruton MN. 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. South African National Scientific Programmes Report no. 144. Pretoria: Council for Scientific and Industrial Research.

Dionne M. 1985. Cannibalism, food availability and reproduction in the mosquitofish (*Gambusia affinis*): a laboratory experiment. *American Naturalist* 126: 16–23.

Ellender B, Weyl OLF. 2014. A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions* 2: 117–132.

Fernández-Delgado C. 1989. Life history patterns of the mosquitofish *Gambusia affinis* in the estuary of Guadalquivir River of south-west Spain. *Freshwater Biology* 22: 395–404.

Fernández-Delgado C, Rossomanno S. 1997. Reproductive biology of the mosquitofish in a permanent natural lagoon in south-west Spain: two tactics for one species. *Journal of Fish Biology* 51: 80–92.

Foxcroft LC, McGeoch M. 2011. Implementing invasive species management in an adaptive management framework. *Koedoe* 53: Art. #1006.

Fraile B, Sáez FJ, Vicentini CA, González A, Miguel MP, Paniagua R. 1994. Effects of temperature and photoperiod on the *Gambusia affinis holbrooki* testis during spermatogenesis period. *Copeia* 1994: 216–221.

Garcia-Berthou E. 1999. Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology* 55: 135–147.

Haynes JL. 1993. Annual reestablishment of mosquitofish population in Nebraska. *Copeia* 1993: 232–235.

Haynes JL, Cashner RC. 1995. Life history and population dynamics of the western mosquitofish: a comparison of natural and introduced populations. *Journal of Fish Biology* 46: 1026–1041.

Howard-Williams C, Liptrot MRM. 1980. Submerged macrophyte communities in a brackish South African estuarine-lake system. *Aquatic Botany* 9: 101–116.

Howell DH, Woodford DJ, Weyl OLF, Froneman W. 2013. Population dynamics of the invasive *Gambusia affinis* in irrigation impoundments of the Sundays River valley, Eastern Cape, South Africa. *Water SA* 39*:* 485–490.

Hughes LA. 1985. Seasonal changes in fecundity and size at first reproduction in an Indiana population of mosquitofish *Gambusia affinis*. *American Midland Naturalist* 114: 30–36.

Hurlbert SH, Zedler J, Fairbanks D. 1972. Ecosystem alteration by mosquitofish (*Gambusia affinis*) predation. *Science* 175: 639–641.

Jobling M. 1981. Temperature tolerance and the final preferendum – rapid methods for the assessment of optimum growth temperatures. *Journal of Fish Biology* 19: 439–455.

Jones RW, Weyl OLF, Swartz ER, Hill MP. 2013. Using a unified invasion framework to characterize Africa's first loricariid catfish invasion. *Biological Invasions* 15: 2139–2145.

Krumholz LA. 1948. Reproduction in the western mosquitofish, *Gambusia affinis affinis* (Baird and Girard), and its use in mosquito control. *Ecological Monographs* 18: 1–43.

Leyse KE, Lawler SP, Strange T. 2004. Effects of an alien fish, *Gambusia affinis*, on an endemic California fairy shrimp, *Linderiella occidentalis*: implications for conservation of diversity in fishless waters. *Biological Conservation* 118: 57–65.

Linder HP, Johnson SD, Kuhlmann M, Matthee CA, Nyffeler R, Swartz ER. 2010. Biotic diversity in the southern African winter rainfall region. *Current Opinion in Environmental Sustainability* 2: 109–116.

Lloyd L. 1986. An alternative to insect control by "mosquitofish", *Gambusia affinis*. *Arbovirus Research Australia* 1986: 156–163.

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. 100 of the world's worst invasive alien species: a selection from the Global Invasive Species Database. Auckland: Invasive Species Specialist Group.

Maglio VJ, Rosen DE. 1969. Changing preferences for substrate colour by reproductively active mosquito fish *Gambusia affinis* (Baird and Girard) (Poeciliidae: Atheriniformes). *American Museum Novitates* 2397: 1–39.

Marchetti MP, Moyle PB, Levine R. 2004. Alien fishes in California watersheds: characteristics of successful and failed invaders. *Ecological Applications* 14: 587–596.

Meffe GK, Crump ML. 1987. Possible growth and reproductive benefits of cannabilism in the mosquitofish. *American Naturalist* 129: 203–212.

Miura T, Takashi RM, Steward RJ. 1979. Habitat and food selection by the mosquitofish *Gambusia affinis*. *Proceedings of the California Mosquito Vector Control Association* 47: 46–50.

Nordlie FG. 2006. Physicochemical environments and tolerances of cyprinodontoid fishes found in estuaries and salt marshes of eastern North America. *Reviews in Fish Biology and Fisheries* 16: 51–106.

Olds AA, Smith MKS, Weyl OLF, Russell IA. 2011. Invasive alien freshwater fishes in the Wilderness Lakes System, a wetland of international importance in the Western Cape Province, South Africa. *African Zoology* 46: 179–184.

Pen LJ, Potter IC. 1991. Reproduction, growth and diet of *Gambusia holbrooki* (Girad) in a temperate Australian river. *Aquatic Conservation: Marine and Freshwater Ecosystems* 1: 159–172.

Pérez-Bote JL, López MT. 2005. Life-history pattern of the introduced eastern mosquitofish, *Gambusia holbrooki* (Baird and Girard, 1854), in the Mediterranean-type river: the River Guadiana (SW Iberian Peninsula). *Italian Journal of Zoology* 72: 241–248.

Pyke GH. 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Reviews in Fish Biology and Fisheries* 15: 339–365.

Pyke GH. 2008. Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. *Annual Review of Ecology, Evolution and Systematics* 39: 171–191.

Pyke GH, White AW. 2000. Factors influencing predation on eggs and tadpoles of the endangered green and golden bell frog *Litoria aurea* by the introduced plague minnow *Gambusia holbrooki*. *Australian Zoologist* 31: 496–505.

Randall RM. 1990. Information sheet for the site designated to the list of wetlands of international importance in terms of the Convention on Wetlands of International Importance especially as Waterfowl Habitat. Available at https://rsis.ramsar.org/RISapp/files/RISrep/ZA524RIS.pdf [accessed 3 March 2015].

Russell IA. 1999. Changes in the water quality of the Wilderness and Swartvlei lake systems, South Africa. *Koedoe* 42: 57–72.

Russell IA. 2013. Spatio-temporal variability of surface water quality parameters in a South African estuarine lake system. *African Journal of Aquatic Science* 38: 53–66.

Snelson FF. 1989. Social and environmental control of life history traits in poeciliid fishes. In: Meffe GK, Snelson FF (eds), *Ecology and evolution of livebearing fishes*. Upper Saddle River: Prentice Hall. pp 149–161.

Spear D, McGeoch MA, Foxcroft LC, Bezuidenhout H. 2011. Alien species in South Africa's national parks. *Koedoe* 53: Art. #1032.

Stearns SC, Sage RD. 1980. Maladaption in a marginal population of the mosquito fish *Gambusia affinis*. *Evolution* 34: 65–75.

Trendall JT. 1982. Covariation of the life history traits in the mosquitofish, *G. affinis*. *American Naturalist* 119: 774–783.

Tweddle D, Bills R, Swartz E, Coetzer W, Dacosta L, Engelbrecht J et al. 2009. The status and distribution of freshwater fishes. In: Darwall WRT, Smith KG, Tweddle D, Skelton P (eds), *The status and distribution of freshwater biodiversity in southern Africa*. Gland: IUCN; Grahamstown: SAIAB.

Uliano E, Cataldi M, Carella F, Migliaccio O, Laccarino D, Agnisola C. 2010. Effects of acute changes in salinity and temperature on routine metabolism and nitrogen excretion in gambusia (*Gambusia affinis*) and zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology* 157: 283–290.

Vondracek B, Wurtsbaugh WA, Cech JJ. 1988. Growth and reproduction of the Mosquitofish, *Gambusia affinis*, in relation to temperature and ration level: consequences for life history. *Environmental Biology of Fishes* 21: 45–57.

Weisser PJ, Howard-Williams C. 1982. The vegetation of the Wilderness Lakes system and the macrophyte encroachment problem. *Bontebok* 2: 19–40.

Whitfield AK, Allanson BR, Heinecken TJE. 1983. Estuaries of the Cape. Part II. Synopses of available information on individual system. Report no. 22 Swartvlei (CMS 11). Council for Scientific and Industrial Research Report no. 421. Stellenbosch: Council for Scientific and Industrial Research.

Received 30 July 2014, accepted 19 November 2014
Associate Editor: Theresa Wossler